Constitutive Regulation of P450s by Endocrine Factors

References:


<table>
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<tr>
<th>Substrate</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP1A2</th>
<th>CYP2E1</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
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<td>(Pharmacol Ther 44, 147-55, 1989)</td>
<td>(n = 50)</td>
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<td>(Br J Clin Pharmacol 37:563-9, 1994)</td>
<td>(n = 36)</td>
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<td>(Br J Clin Pharmacol 39:511-8, 1995)</td>
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<td>(Pharmacogenetics 4:109-16, 1994)</td>
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<td>Pharmacogenetics, 11:1-11, 2001</td>
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<td>(n – 137)</td>
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All values represent CL/F for oral dose; analogous to hepatic intrinsic clearance when Fa = 1.
Sources of Interindividual Variability in P450 Expression

- Genetic Constitution
- Diet
- Smoking
- Age
- Sex
- Pregnancy
- Exercise
- Starvation
- Infection
- Hormones
- Cytokines
- Paracrine Factors

Adapted from Vessell, 1981
Transcriptional Control of P450 Levels

• Nearly all of the major human P450 genes appear to be under some level of basal transcriptional control.

• Transcription factors binding to their cognate response elements, directly or indirectly affect the 5’-flanking tertiary structure (looped enhancer-promoter complex), and increase binding/function of polymerase II complex.
A major physiological function of nuclear hormone receptors involves the maintenance of cholesterol/bile acid/vitamin D/fatty acid homeostasis in the liver and possibly other organs (Handschin and Meyer, Arch Biochem Biophys, 2005).

Metabolism (Oxidation/Conjugation) and/or Excretion (Transport) Of Endogenous Molecules

Adapted from Handschin and Meyer, 2005
Activation of hPXR by Endogenous Steroids

- It is has been assumed that an endogenous ligand (pregnane?), activates PXR at a relevant circulating concentration; possibly an additive effect of multiple ligands.

Activation of (PXRE)_3-LUC reporter by 50 µM of indicated steroid (A) or 10 µM individual and 100 µM steroid cocktail mix (B). *Genes & Development* 12:3195-3205, 1998
Do Bile Acids Activate CYP3A4?

- Bile acids are ligands for mPXR and hPXR, but may only induce the mouse CYP3A enzymes, except in cholestasis (*Dussault et al.*, *PNAS*, 2003).

- However, CYP3A4 catalyzes 6α- and 4β-hydroxylation of cholesterol (triol→tetrol); an important detoxification process (*Bodin et al.*, *JBC*, 2002).
Up- and Down-Regulation of PXR by GC and IL-6

- A number of cytokines have been shown to down-regulate CYP expression in cultured hepatocytes, and some exert effects in vivo. (Mol Pharmacol 44:707, 1994; JPET 285:127-34, 1998)

Studies from Pascussi et al., suggest CYP3A4 may be regulated by alteration of PXR expression.
Regulation of PXR and CYP3A4 by Glucocorticoids

Induction of hPXR by pre-treatment of hepatocytes with dexamethasone (1 µM)

Enhancement of CYP3A4 induction by pre-treatment of Hepatocytes with Dex (an indirect, PXR induction, GR-mediated effect?)

*Mol Pharmacol* 58:361-72, 2000
GH Signaling Pathway – Effects on Hepatic CYP2C

- GH secretion patterns control the hepatic expression of male-specific CYP2C isozymes in the rat.

(G AS (γ-interferon activated sequence) motif regulated by STAT homo- or heterodimers: TTC(N)₃GAA

• Humans don’t show the same extreme sexual dimorphism in GH secretion and P450 expression, but there is evidence for GH transcriptional control (Waxman and Holloway, Mol Pharmacol 76:215-28, 2009).
CYP3A4 Transgenic Mice

- CYP3A4 suppressed by pulsatile GH secretion (male pattern); make explain lower hepatic CYP3A4 activity in men, vs women.

Up-regulation of CYP3A4 by GH and T₃ in Hepatocytes

CYP3A4 is up-regulated in cultured human hepatocytes by a physiologically relevant concentration of circulating hormones. Dex effect mediated by PXR induction (see below).

CYP3A4 has a GAS element in the 5’-flanking region but it is not clear if this is functional; GH may mediate its effects indirectly through changes in NRs (e.g., HNFα, PXR)

Human hepatocytes treated continuously from day-1 with indicated hormone (Dex, 10 nM; T₃, 1 nM; GH, 100 ng/mL

Women of reproductive age tend to have a higher baseline and a greater average pulse amplitude than men – affects growth characteristics and possibly hepatic CYPs.
Gender Differences in Hepatic CYP3A Activity

Oral Midazolam Dose (0.028 mg/kg)

- The 4-hour MDZ concentration is inversely proportional to oral clearance (Lin et al., Pharmacogenetics 11:781-91, 2001)
- Other supporting references (Gorski et al., CPT 64:133-43, 1998).

Higher CYP3A Activity in Women
Transcriptional Activation of *CYP3A* Promoters by 1,25-D$_3$ in Transiently Transfected LS180 Cells

**Graph:**
- SV40-Luc
- TK-LUC
- 3AP1
- 3A23
- 3A4

**Fold-Increase over Untreated Control**

**Legend:**
- SV40
- TK
- PXRE
- DR3
- 3AP1
- 3A23
- 3A4

**Annotations:**
- **CYP3A4:** ER-6 (PXRE)
- **CYP3A23:** ER-6 (PXRE) and DR-3
- **CYP3AP1:** nonfunctional ER-6

**References:**
*Thummel et al., Mol Pharmacol 2001*
Activation of CYP3A4 PXRE by 1,25-D$_3$ and hVDR/RXR$\alpha$

**Graph**

- **pcDNA3**: 100
- **hVDR**: 80

**COS-7 cells co-transfected with hVDR and CYP3A4 ER6-CAT constructs.**

1,25-D$_3$ activated gene transcription only in the presence of hVDR. The PXR ligand, rifampin, had no effect, as expected.
Ratio of Relevant [mRNA] in Paired Human Jejuna/Liver

- ~ 50-fold higher levels of VDR mRNA in human intestine, compared to liver

- CYP3A4, MDR1 and PXR mRNA contents are also higher in the intestine, in contrast to RXRα.

Xu et al., Mol Pharmacol, 2006
LS180 Cells: A Model for Human Enterocytes

- LS180 cells contain relative high expression of hPXR, VDR, CYP3A4 and TRPV6, compared to Caco-2 cells (low PXR and minimal constitutive CYP3A4 expression).

Low dose $1\text{,}25(\text{OH})_2\text{D}_3$ induces all 3 VDR gene targets.

Rifampin is a selective CYP3A4 inducer.
Heterogeneous Distribution of CYP3A4 in Small Intestine

Although there is significant inter-individual variability, CYP3A4 protein declines by 50%, on average D→I.

Pattern may be Vitamin D dependent.
• Marked preferential expression of known VDR target genes (TRPV6 and calbindin D9K) in the upper small intestine, suggests that VDR signaling (and any effects on CYP3A4) is restricted to the upper region of the small intestine.

Zheng et al., Journal of Steroid Biochemistry and Molecular Biology, 2012
VDR expression appears to be relatively constant along the length of the small intestine.

What about delivery of the ligand?
Alternative Mechanisms of 1,25-D$_3$ Delivery and Regulation of Intestinal CYP3A4

- **Blood**: Renal CYP27B1, Hepatic CYP27A
- **Enterocyte**: VDR, RXR, ER6, CYP3A4, UGT 1A4/SULT2A1
- **GI Lumen**: bile

1,25-D$_3$ -> Liver -> 1,25-D$_3$-G/S

1,25-D$_3$ -> Blood

1,25-D$_3$ -> Enterocyte

1,25-D$_3$ -> GI Lumen
Keto Bile Acids Activate VDR and CYP3A4 Expression

Some keto-bile acids may also be important and variable regulators of intestinal and possibly hepatic CYP3A4 expression. However, these molecules are highly insoluble and conjugated to taurine etc.

Science 296:1313-6, 2002